circulating ACE activity and to hypertrophic cardiomyopathy, heart failure in general and maybe also to myocardial infarction (MI). This marker is a deletion (D) or insertion (I) of a gene sequence. This generates three different genotypes II, ID and DD.

Aim of study: To prospectively study ACE gene polymorphism in consecutive acute MI patients and healthy controls both groups without upper age limits.

Results: The genotype frequency is presented in the table. The D allele had approximate frequency of 0.52 in the control group and 0.58 in the acute MI group. The DD/II ratio was higher in the acute MI group ($p < 0.05$). Odds ratio for the DD genotype was 1.35 (confidence interval 0.94–1.97).

ACE genotype	II %	ID %	DD %
Acute MI patients (n = 180)	35 (19)	80 (45)	65 (36)
Healthy controls (n = 430)	112 (26)	192 (45)	126 (29)

Conclusion: The approximate allele frequency as well as odds ratio of the DD genotype is in our study in accordance with other findings (0.53–1.34, Cambien et al, Nature 1992;359:641). ACE gene polymorphism is related to acute MI, its importance in relation to other risk factors needs to be assessed.

4:15

766-2 In Vivo Measurement of Myocardial Gene Expression in the Human Heart

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We have previously described a quantitative method for measuring mRNA abundance in endomyocardial biopsy-sized pieces of human heart. This method, reverse transcription-quantitative PCR (RT-QPCR), uses a known amount of *in vitro* transcribed internal standard to generate simultaneously amplified unknown and standard products in the same amplification reaction. We now describe the use of this method to actually measure mRNA abundance for genes of interest in endomyocardial biopsy specimens removed from subjects with either idiopathic dilated cardiomyopathy (IDC) and systolic dysfunction (LVEF = 16.8%, RVEF = 27.7%) or primary pulmonary hypertension (PPH) and right ventricular hypertrophy. (Results are in molecules mRNA $\times 10^5/\mu g$ total RNA ± SEM)

Group	β_1-adrenergic receptor	β_2-adrenergic receptor	Atrial natriuretic peptide	α-myosin heavy chain
IDC (n = 5)	0.44 ± 0.31	3.29 ± 1.04	23.4 ± 12.4	18.3 ± 4.61
PPH (n = 3)	0.29 ± 0.08	5.93 ± 1.29	58.2 ± 20.3	10.7 ± 3.44

Conclusions:

1. Gene expression can be measured *in vivo* in endomyocardial biopsies using RT-QPCR.
2. Using 3–6 endomyocardial biopsies we estimate that mRNA abundance can be measured for as many as 30 genes of interest.
3. Concentrically hypertrophied myocardium may have a different gene expression pattern than failing myocardium.

4:30

766-3 Fingerprinting Analysis of mRNA from Restenotic and Primary Atherosclerotic Coronary Plaque Material Obtained by Atherectomy

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In order to compare the expression of gene products in coronary artery restenotic (R) lesions versus that in primary atherosclerotic (A) lesions, we performed RNA fingerprinting of 6 R and 20 A tissue samples obtained by directional coronary atherectomy. Poly A^+ RNA was extracted and cDNA was produced using AMV reverse transcriptase. Priming was performed using either oligo dT or an arbitrary primer for first strand synthesis. Arbitrarily-primed PCR (AP-PCR) amplification of the resultant cDNA was performed using either a single arbitrary 17-mer or a combination of 2 arbitrary 20-mers as primers. PCR conditions were adjusted to enhance arbitrary priming and included a high initial Mg^{++} concentration (4 mM), high initial primer concentration (10 μM), and low annealing temperature (40°C). Subsequent cycles (total of 40) were performed under high stringency conditions and included 10 μCi of dCTP. The PCR products were separated on a 4.5% sequencing gel and subjected to autoradiography, revealing >50 bands of ≈100–500 bp in size. Two products were expressed at higher levels in R than A samples. These products were reamplified using the original primers, TA cloned and sequenced, revealing fibronectin and a unique gene product not found in GenBank. Our results suggest that: (1). most gene products in R and A samples are identical, (2) fibronectin levels are increased in R samples, and (3) the AP-PCR method will be useful to identify novel differentially expressed gene products. This is the first use of the AP-PCR method to compare gene expression in restenotic and primary atherosclerotic lesions.

4:45

766-4 X-Linked Cardiomyopathy: Selective Deficiency of Dystrophin mRNA and Protein in the Heart

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Mutations in the membrane associated cytoskeletal protein dystrophin is typically associated with Duchenne and Beckers Muscular Dystrophy. In some cases dystrophin mutations appear causes a selective cardiomyopathy with little skeletal muscle disease. The molecular mechanisms which cause this phenotype have not been defined. We are particularly interested in how a mutation in a protein normally expressed in all muscle tissues can selectively cause disease in the heart. Most cardiomyopathic dystrophin mutations occur at the 5′ end of the gene, suggesting that regulatory elements may play a role in the cardiac specific phenotype. We have analyzed cardiac and skeletal muscle dystrophin mRNA and protein from a family with severe cardiomyopathy, and a 5′ duplication in exons 2–7 of the dystrophin gene. Sequence analysis of cDNA across the mutation site, showed no difference between heart and skeletal muscle transcripts. However, ribonuclease protection assay demonstrated markedly diminished dystrophin transcript in the heart. Western blot and immunocytochemistry showed marked reduction in cardiac dystrophin protein compared with skeletal muscle. These results demonstrate that mutations in the 5′ dystrophin gene can cause cardiomyopathy without significant muscle disease by a selective reduction of dystrophin gene expression in the heart.

5:00

766-5 Depressed Intracellular Calcium Homeostasis and Mechanical Properties of Cardiomyocytes in Annexin VI Transgenic Mouse

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Annexins are a unique family of calcium-dependent, phospholipid-binding proteins found in various tissues. Annexin VI, a major member in the family, exists in the heart and acts as a potent regulator of the sarcoplasmic reticulum calcium-release channel, cardiac L-type calcium channel and Na^+/Ca^{2+} exchanger. To investigate the role of the regulation of annexin VI in intracellular calcium homeostasis and mechanical properties of cardiomyocytes, we used the transgenic mice which overexpresses annexin VI (TG, n = 6) and control mice (C, n = 6) at three months of age. A 10-fold increase in annexin VI was revealed by Western blotting in TG mice compared to the controls. No differences were found between TG and C groups in heart/body wt., lung/body wt. or myocyte size.

Cardiomyocytes from TG and C mice were isolated and field paced at 0.25, 0.5 and 1.0 Hz (15, 30 and 60 beats per minute). The myocytes of TG mice demonstrated significant decreases in shortening fraction (% shortening, TG vs C, 4.59 ± 0.42 vs 8.62 ± 0.85, $P < 0.01$), rate of shortening (+dl/dt, μm/sec, TG vs C, 93.54 ± 24.31 vs 148.77 ± 20.23, $P < 0.05$) and rate of relengthening (−dl/dt, μm/sec, TG vs C, 59.28 ± 8.32 vs 119.66 ± 15.94, $P < 0.01$) at 1.0, but not at 0.25 or 0.5 Hz stimulation. FURA-2 loaded myocytes from TG mice showed lower baseline levels of calcium at all three stimulation frequencies respectively (340/380 ratio, TG vs C, 0.526 ± 0.075 vs 0.968 ± 0.550 at 0.25 Hz, $P < 0.001$; 0.566 ± 0.044 vs 0.982 ± 0.050 at 0.5 Hz, $P < 0.001$; 0.564 ± 0.043 vs 1.016 ± 0.051 at 1.0 Hz, $P < 0.001$). The amplitude of calcium signals decreased in annexin VI TG mice at 0.25 Hz (340/380 ratio, TG vs C, 0.974 ± 0.148 vs 0.541 ± 0.082, $P < 0.05$) and at 0.5 Hz stimulation (0.858 ± 0.141 vs 0.503 ± 0.077, $P < 0.05$). The duration of calcium signals was attenuated in TG mice compared to control at 0.25 Hz (sec, 1.033 ± 0.227 vs 1.507 ± 0.150, $P < 0.05$).

We conclude that overexpression of annexin VI in mouse myocytes causes depression in the intracellular free calcium concentration, and frequency-dependent depression in the extent and rate of the shortening and relengthening.